AD_____

GRANT NUMBER DAMD17-94-J-4474

TITLE: Sequence Specific and Synergistic Binding of Drugs to DNA

PRINCIPAL INVESTIGATOR: Fu-Ming Chen, Ph.D.

CONTRACTING ORGANIZATION: Tennessee State University
Nashville, Tennessee 37209-1561

REPORT DATE: October 1997

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1997	Annual (23 Sep 96 - 22 Sep 97)

4. TITLE AND SUBTITLE
Sequence Specific and Synergistic Binding of Drugs to DNA

5. FUNDING NUMBERS
DAMD17-94-J-4474

6. AUTHOR(S)
Fu-Ming Chen, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Tennessee State University
Nashville, Tennessee 37209-1561

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

Our goals were to study the sequence specific and synergistic binding of three drugs having distinctly different binding modes: actinomycin D, a guanine specific intercalator; chromomycin A_3, a guanine specific minor groove binder; and distamycin A (DST), an A•T specific groove binder. It is essential that characteristics such as binding affinities, sequence specificities, and kinetic behaviors of each drug be understood prior to synergistic studies. Uncovering sequence specificity of DST at the tetranucleotide level and spectral techniques for differentiating its1:1 and 2:1 binding modes was our main focus during the past year. DST binding studies were made with self-complementary decamers of the form d(GCG-X-CGC), where X = AATT, ATAT, TATA, or TTAA, and with non-self-complementary decamers of the form d(GCG-X-GCG)•d(CGC-Y-CGC), where X = ATTA, ATTT, TATT, TTAT, TTTA, or TTTT, and Y is complementary to X. It was found that ATTT/TAAA and TTTT/AAAA binding sites exhibit significantly stronger DST affinities than the other sequences studied. To investigate the spectral characteristics of 2:1 complexes, studies were also made with 11-mers containing a 5-base-paired site, ATATA/TATAT or AAGTT/AACTT. The distinct circular dichroic characteristics exhibited by the 1:1 and 2:1 complexes may provide diagonistic potentials for binding mode differentiation for this drug.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer	15
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

✓ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Jacqueline Chen 10-21-97

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION

Combination chemotherapy is one of the important strategies in cancer treatments. This is based on the observation that administering certain drugs together is more effective than giving individual drugs separately. Although the reason for such an effect is not understood, it may be related to the synergistic effect of their binding to biomacromolecules. Consequently, studies on the interplay among drugs capable of binding to different regions of DNA will be of considerable interest. Understanding the synergism of drugs at the molecular level may have important implication for designing more effective chemotherapeutic strategies in breast cancer treatments. Our proposal focuses on the sequence specific binding and synergistic effect of three drugs having distinctly different binding modes: actinomycin D (ACTD), a guanine specific intercalator; chromomycin A_3 (CHR), a guanine specific minor groove binder; and distamycin A (DST), an A•T specific groove binder. In order to investigate the possible synergistic effects of drugs on DNA binding, it is essential that binding characteristics of each individual drug such as binding affinities, sequence specificities, and kinetic behaviors be thoroughly elucidated. Consistent witn the Statement of Work outlined in the proposal, our laboratory has focused on the sequence specificity and binding mode studies of DST during the past year.

Sequence-specific recognition of DNA molecules by proteins and small molecules is an important component in the regulation of many biological processes. Understanding the structure, sequence specificity, and forces responsible for the binding of antibiotics to DNA molecules is an important first step in the design of new drugs and sequence-specific probes. Two related antibiotics, netropsin (NET) and distamycin A (DST), have received considerable attention as models of sequence-specific, non-intercalative DNA binding molecules. DST and NET are oligopeptides which are potent antibacterial, antiviral, and antineoplastic agents whose pharmacological activity is correlated to DNA binding (Hahn, 1975). These two antibiotics form non-covalent complexes with duplex DNAs with preference for AT-rich domains such as the promoter regions. For this reason, the antibiotics act as template poisons and inhibit DNA-dependent DNA and RNA polymerase reactions. Both DST and NET show considerable DNA binding affinities, yet their binding does not involve intercalation but occurs outside the helix in the minor groove (Luck et al., 1977). For the most part, physical studies until recently have focused on the smaller netropsin molecule, and the results from such studies have been extended by analogy to the larger distamycin molecules.

DST was discovered in 1958 but systematic studies on its molecular pharmacology did not begin until 1969. Recent years have seen the upsurge of interest on this drug as a model for the DNA minor groove binding of A:T-specificity. Such interests were further fueled by the recent finding of a 2:1 mode of drug binding on some base sequences. Several studies of 1:1 DST-DNA complexes have provided insights into both the specificity and the forces responsible for the tight binding of this drug. The structure of the DST-d(CGCGAATTCGCG)$_2$ complex was determined by a combination of 2D NMR experiments and molecular mechanics calculations (Pelton & Wemmer, 1988). It was found that the minimal binding site consists of just four A:T base pairs and that DST fits snugly into the 5'-AATT-3' minor-groove binding site. This complex is characterized

by van der Waals contacts between adenine C2H and drug H3 protons, potential three-center hydrogen bonds between drug amide and adenine N3 and thymine O2 atoms that protrude from the minor groove, and stacking of DNA O1' atoms over each of the three pyrrole rings. The first two rings of the drugs are approximately parallel, while the third ring is turned to conform to the rotation of the helix. No large structural changes were observed for the DNA. It was suggested that the dipole-induced dipole interactions of the sugar O1' atoms and the three N-methylpyrrole rings contribute to the stability of the complex and provide a molecular basis for the fact that binding is enhanced by the addition of N-methylpyrrole rings. Subsequent calorimetric studies (Breslauer et al., 1987) have shown that DST binds tightly to the same four-base site within the sequence $d(GCGAATTCGC)_2$, with a binding constant of $2.7 \times 10^8\ M^{-1}$. This is in striking contrast to the binding constant of $2 \times 10^5\ M^{-1}$ reported for the 5'-TATA-3' site within the sequence $d(GGTATACC)_2$, obtained by quantitative analysis of footprinting data. Although the discrepancies may partly be attributed to the oligomeric length and different experimental techniques employed, sequence specificity of this drug may also be responsible. The possible sequence specificity of this drug is further underscored by a recent crystallographic studies of DST with $d(CGCAAATTTGCG)_2$ (Coll et al., 1987) revealing that the drug bound to a 5'-ATTT-3' sequence, although other sites with four A:T base pairs were available.

Most interestingly, recent NMR studies have further indicated that binding sites of at least five base pairs in length can accommodate two DST molecules side-by-side in an antiparallel orientation (Pelton & Wemmer, 1989, 1990). In this 2:1 complex, each ligand preserves all the molecular recognition elements of minor groove binders: electrostatic interactions with the negative potential in the groove of DNA, specific hydrogen bonds of the ligand amide protons with acceptor atoms of the bases, and van der Waals interactions with the wall of the minor groove (Zimmer & Wahnert, 1986). The ease of forming a 2:1 DST:duplex complex vs. a 1:1 DST:duplex depends highly on the DNA sequence. While DST binds exclusively as a dimer to AAGTT/AACTT and ATATA/TATAT even at low [ligand]/[duplex] ratios, only 1:1 DST complexes are observed with AAAAA/TTTTT at [ligand]/[duplex] ratios up to 1:1. At higher ratios 2:1 DST complexes form with AAAA/TTTT also (Wemmer et al., 1994). The AAATT site is intermediate in its cooperativity for DST binding. These data suggest that the binding mode of DST is determined by both sequence-dependent groove width and flexibility. Indeed, the cooperativity of 2:1 DST complex formation has been used to probe sequence-dependent variations in minor groove width (Fagan & Wemmer, 1992; Wemmer et al., 1994). In contrast to DST, the dication netropsin binds only as a single molecule per binding site, suggesting that the side-by-side arrangement of two NET ligands in the minor groove is inhibited by charge interactions.

These studies reinforce the importance of base sequence on the formation of both 1:1 and 2:1 complexes. Understanding the factors which govern the formation of these two types of complexes will, thus, be of value in discovering new sequence-specific ligands which are optimized for binding in specific modes to specific sequence targets. Unfortunaately, there have been no systematic studies on the sequence specific binding on the A:T-containing sequences, aside from the NMR studies carried out thus far. To remedy the situation, our objective last year was to carry out systematic sequence-specific

binding studies utilizing optical techniques and oligonucleotides containing DST binding sites of 4 and 5 base pairs.

BODY

Experimental methods, assumptions, and procedures.

As a first step in elucidating the tetranucleotide sequence specificity of DST, binding studies were carried out on self-complementary decamers of the form d(GCG-X-CGC), with X = AATT, ATAT, TATA, and TTAA, and non-self-complementary oligomers of the form d(GCG-X-GCG)/d(CGC-Y-CGC), where X = ATTA, ATTT, TATT, TTAT, TTTA, TTTT, and Y is complementary to X. For binding studies on 2:1 complexes, oligomers of the form d(GCG-X-GCG)•d(CGC-Y-CGC) will be employed, where X = ATATA or AAGTT and Y is complementary to X.

Since previous NMR studies had shown that a 4-base A/T binding site forms a 1:1 complex whereas a 5-base site can also form a 2:1 complex and exclusively forming such dimer complexes at the -ATATA- and -AAGTT- sites. Thus, it was hoped that spectral titrations using these two types of oligomers would provide binding parameters for these two modes of binding and spectral comparison would provide their possible differentiation. To this end, CD spectral measurements were most promising since a dimer formation devoid of a plane of symmetry usually led to the formation of an exciton-type of CD couplet.

Equilibrium Binding Titrations via Absorbance Spectral Changes. DST exhibits an absorbance maximum at 303 nm when free in solutions. Successive additions of DNA lead to slight bathochromic shifts and intensity enhancements near 330 nm. Thus, absorbance changes at 350 nm (to avoid interference from the residual DNA absorbance) were used to construct binding isotherms and to obtain binding parameters. Since most of the Scatchard plots can be approximated by straight lines, the binding parameters were deduced via linear least-squares fits. DNA melting measurements were also carried out to investigate the effect of DST binding on the DNA duplex stability. The extent of melting temperature increase of DNA upon drug binding can also be correlated to the relative binding affinity.

Absorption spectra were measured with a Cary 1 E spectrophotometric system. Spectral titrations were carried out at 25 °C by starting with a drug solution followed by progressive additions of the oligomer stock. Thermal denaturation experiments of 40 μM oligomer in the absence and in the presence of 7 μM DST were carried out with 1-cm semimicro cells by monitoring the absorbance at 275nm. A heating rate of 0.5 °C/min was maintained by the temperature controller accessory. Melting temperatures were deduced via differential melting profiles.

Equilibrium Binding Titrations and Spectral Characterization of DST Binding Modes via Circular Dichroism. DST is not optically active when free in solutions. In the presence of DNA, however, a strong positive CD band with a maximum near 330 nm is induced. Thus, induced CD intensities at 330 nm can be used to construct binding isotherms via spectral titrations. CD spectra were measured at room temperature by a Jasco J-500A recording spectropolarimeter using water-jacketed cylindrical cells of 2-cm

pathlength. CD titrations were carried out with 80 μM DNA nucleotide followed by progressive additions of the drug stock solution. Measurements were made from 300 to 380 nm for spectral titrations. For CD spectral characterization, however, measurements were made from 230 to 380 nm.

Since DST is not very stable in aqueous solutions, stock solutions were prepared immediately before use. Its concentrations were determined using $\varepsilon_{303} = 34{,}000\ M^{-1}cm^{-1}$. Synthetic oligonucleotides were purchased from Research Genetics, Huntsville, AL, and used without further purification. Concentrations of these oligomers (per nucleotide) were determined by measuring the absorbances at 260 nm after melting, with use of extinction coefficients obtained via nearest-neighbor approximation using mono- and dinucleotide values tabulated in Fasman (1975). All experiments were carried out in 10 mM HEPPS [N-(2-hydroxyethyl)piperazine-N'-propanesulfonic acid] buffer solutions of pH 8 containing 0.1 M NaCl and 1 mM $MgCl_2$.

Results and Discussion

Our attempts to utilize the data from the absorbance binding titrations to obtain binding parameter were not entirely successful, despite the investment of considerable efforts. The main reason being that the long wavelength absorption band of DST partially overlap with that of DNA. Thus, the largest spectral changes which occur below 300 nm are being complicated by the spectral contributions due to DNA. In order to avoid the interference due to DNA, spectral changes at 350 nm were employed. Absorbance changes at this wavelength, however, are relatively small and no consistent set of results were obtained. Fortunately, CD intensities of DST induced upon DNA binding are more considerable and their values can more reliably be used to obtain binding parameters which are summarized in Table 1 and to be described in this report.

DST Binding to Self-Complementary Tetranucleotide Sequences. No significant differences in the DST binding affinities were found for the self-complementary decameric duplexes studied. The observed binding constants range from 1.3 to $4.5x10^6$ M^{-1}, with $d(GCG\text{-}AATT\text{-}CGC)_2$ exhibiting the highest DST binding affinity and forming an 1:1 complex. This finding apppears to be supported by the thermal denaturation measurements indicating the -AATT-containing decamer exhibits the largest melting temperature increase upon DST binding ($\Delta t_m = 11\ ^{\circ}C$). Our results, thus, suggest the following DST binding order: -AATT- > -TATA- ≥ -ATAT-, -TTAA-.

DST Binding to Non-self-complementary Tetranucleotide Sequences. Of the non-self-complementary tetranucleotide binding sequences studied, -ATTT- and -TTTT- appear to form 1:1 complexes and exhibit considerably higher DST affinities than the other sequences studied, with binding constants of 1.7×10^7 and 1.3×10^7 M^{-1}, respectively. The other sequences studied exibit binding constants ranging from 0.7 to $1.7x10^6$ M^{-1}. The binding order is found to be: -ATTT- ≥ -TTTT- >> -TTAT- ≥ -TATT- ≥ -TTTA-.

CD spectral Characterization of 1:1 and 2:1 DST:Duplex Complexes. CD spectral characteristics are compared in Figure 1 for d(GCG-ATTA-GCG)[D] and d(GCG-AAGTT-GCG)[D] in the presence of increasing amounts of DST, where [D] designates the heteroduplex. It is apparent that the two modes of binding induce distinctly different CD spectral characteristics. DST binding to the ATTA-containing decamer result in the

progressive CD intensity enhancement of the 330-nm band, with surprisingly little spectral alterations below 300 nm. The maintenance of an isoelliptic point near 240 nm suggests a 2-component process. In contrast, DST binding results in a distinctly different spectral alterations. In addition to a significantly larger intensity enhancement of the 330-nm band, gross spectral alterations were seen in the spectral region below 300 nm. Noteworthies are the appearance of a negative minimum near 300 nm and the presence of isoelliptic points near 240, 285, and 305 nm. The presence of isoelliptic points again signify the 2-component process, with the bound species being the 2:1 complex exclusively.

The CD spectral alterations of these two binding modes can more clearly be seen with the difference spectra as shown in Figure 2, where the DNA spectral contributions have been subtracted out. As is apparent, that in addition to the 330 nm band a large positive CD band is induced near 260 nm upon DST binding with a magnitude comparable or larger than that of the 330-nm band. The three isoelliptic points and the induction of a negative CD band near 293 nm can more clearly be seen in the difference spectral presentation.

The observation of distinctly different CD spectral characteristics induced by DST binding at the -ATTA- and -AAGTT- sites, with much larger alteration for the latter, is consistent with the 2:1 complex formation at the 5-base binding site. An exciton-type CD couplet is to be expected if the drug dimer bound to the DNA minor groove is devoid of a plane of symmetry, such as antiparallel orientations with only C_2 symmetry. Indeed, the dramatic positive CD intensity enhancements at 330 and 260 nm accompanied by the appearance of negative maximum near 293 nm and large negative intensity enhancement near 230 nm are consistent with the positive-negative exciton couplets centered near 305 and 240 nm absorbance maxima, respectively. The small negative maximum near 293 nm can be seen as the consequence of partial cancellation of these two couplets, where the negative band of the 305 nm couplet is being partially neutralized by the positive wing of the couplet centering around 240 nm.

Our finding of strong DST binding at the -ATTT- site appears to be consistent with a recent crystallographic study of DST with the symmetric dodecamer $d(CGCAAATTTGCG)_2$ revealing that the drug bound to only one site, namely 5'-ATTT-3', although other sites such as AATT and AAAT were available (Coll et al., 1987). Both 1:1 and 2:1 binding modes, however, have been observed for the binding of DST to this same sequence in an NMR solution study (Pelton & Wemmer, 1990). The data obtained on the 2:1 complexes are consistent with a model in which each drug slides between 5'-AATT-3' and 5'-ATTT-3' binding sites associated with each strand of the duplex at a rate that is fast on the NMR time scale.

An alternative technique for distinguishing 1:1 from the 2:1 complexes of DST may be the kinetic measurements. Since the 2:1 complex formation is likely sequential and requires the widening of minor groove, it is reasoned that both the association and dissociation kinetics will be considerably slower than those of the 1:1 complexes. Indeed, our preliminary stopped-flow kinetic measurements have borne out these predictions. For example, the dertergent-induced dissociation time is around 5 s at 20 ^{o}C for a 2:1 complex, whereas that of 1:1 complex is less than 1 s. More workd need to be done in this area.

Another immediate extension of the current work may be the investigations of other 5-base binding sites, such as -AAATT-, which may form both 1:1 and 2:1 complexes depending on the [DST]/[DNA] ratios. Thus, sequence-dependent dimer binding and possible binding cooperativity can be elucidated by taking advantage of the distinct CD spectral characteristics of these two complexes.

CONCLUSION

Distamycin A is optically inactive when free in solutions. Binding to a 4-base-paired A/T site results in a 1:1 complex formation which is characterized by a significant positive CD spectral formation near 330 nm with negligible effects on the DNA spectral region. In contrast, binding to a -AAGTT- or -ATATA- site results in a 2:1 complex formation which is characterized by a considerably larger CD intensity enhancement at 330 nm and a comparable or even larger CD spectral alteration near 270 nm as well as the appearance of a negative CD band near 290-nm region. CD spectral titrations indicate that among the 4-bse binding sites, ATTT/TAAA and TTTT/AAAA sequences exhibit the strongest distamycin A binding affinities. Our results, thus, indicate that CD spectral measurements can be used to identify and differentiate the binding modes and to obtain meaningful binding parameters.

REFERENCES

Breslauer, K. J., Remeta, D. P., Chou, W.-Y., Ferrante, R., Curry, J., Zaunczkowski, D., Synder, J. G., & Maeky, L. A. (1987) *Proc. Acad. Sci. U.S.A. 84*, 8922-8926.

Coll, M., Frederick, C. A., Wang, A. H.-J. & Rich, A. (1987) *Proc. Natl. Acad. Sci. USA 84*, 8385-8389.

Fagan, P. A., & Wemmer, D. E. (1992) *J. Am. Chem. Soc.114*, 1080-1081.

Fasman, G. D., Ed. (1975) *CRC Handbook of Biochemistry and Molecular Biology,* 3rd ed., Vol. I. p 589, Chemical Rubber Company Press, Inc., Cleveland, OH.

Hahn, F. E. (1974) In *Antibiotics III.* Mechanism of Action of Antimicrobial and Antitumor Agents, pp 79-100. Springer-Verlag, Berlin, Heidelberg, New York.

Hahn, F. E. (1977) *Pharmac. Ther. A. 1,* 475-485.

Luck, G., Triebel, H., Waring, M., & Zimmer, Ch. (1974) *Nucleic Acids Res. 1*, 503-530.

Luck, G., Zimmer, C., Reinert, K., & Arcamone, F. (1977) *Nucleic Acids Res. 4*, 2655-2670.

Pelton, J. G. & Wemmer, D. E. (1988) *Biochemistry 27*, 8088-8096.

Pelton, J. G. & Wemmer, D. E. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86, 5723-5727.*

Pelton, J. G. & Wemmer, D. E. (1990) *J. Am. Chem. Soc. 112*, 1393-1399.

Wemmer, D. E., Geierstanger, B. H., Fagan, P. A., Dwyer, T. J., Jacobsen, J. P., Pelton, J. G., Ball, G. E., Leheny, A. R., Chang, W.-H., Bathini, Y., Lown, J. W., Rentzeperis, D., Marky, L. A., Singh, S., & Kollman, P. (1994) in *Structural Biology: The State of the Art. Vol 2. Proceedings of the 8th Conversation in the Discipline Biomolecular Stereodynamics* (Sarma, H., & Sarma, M. H., Eds) pp 301-323, Adenine Press, New York.

Zimmer, Ch. (1975) *Prog. Nucleic Acid Res. Molec. Biol. 15,* 285-318.

Zimmer, C., & Wahnert, U. (1986) *Prog. Biophys. Mol. Biol. 47,* 31-112.

Table 1. Comparison of Distamycin A Binding Parameters and Melting Temperatures.

Oligomer	K (μM^{-1})	n (/duplex)	t°_m (°C)	t_m (°C)	Δt_m (°C)
d(GCG-AATT-CGC)$_2$	4.5	1.0	46.0	57.0	11.0
d(GCG-ATAT-CGC)$_2$	1.3	1.4	46.5	51.5	5.0
d(GCG-TATA-CGC)$_2$	1.7	1.6	45.5	51.8	6.3
d(GCG-TTAA-CGC)$_2$	1.3	1.6	47.0	51.5	4.5
d(GCG-ATTT-GCG)•d(CGC-AAAT-CGC)	17	0.8	48.0	55.5	7.5
d(GCG-TATT-GCG)•d(CGC-AATA-CGC)	1.1	1.2	43.5	52.5	9.0
d(GCG-TTAT-GCG)•d(CGC-ATAA-CGC)	1.7	1.6	42.0	48.5	6.5
d(GCG-TTTA-GCG)•d(CGC-TAAA-CGC)	0.7	1.3	39..0	45.5	6.5
d(GCG-TTTT-GCG)•d(CGC-AAAA-CGC)	13	0.9	46.0	55.0	9.0
d(GCG-ATATA-GCG)•d(CGC-TATAT-CGC)	9.1	1.7	43.0	54.0	11.0
d(GCG-AAGTT-GCG)•d(CGC-AACTT-CGC)	6.7	1.8	48.5	57.5	9.0

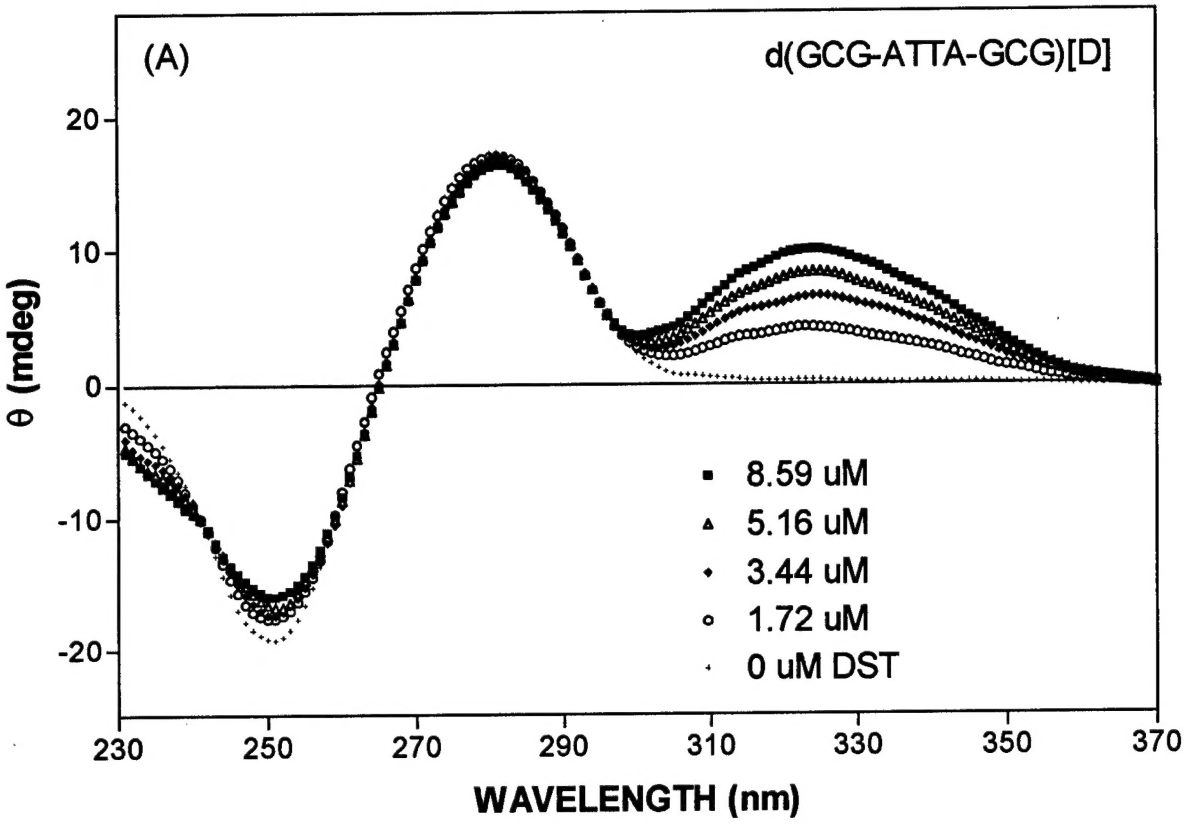
t°_m and t_m are melting temperatures of 40 μM (base) oligomeric duplexes in the absence and in the presence of 7 μM DST, respectively.

FIGURE LEGENDS

Figure 1. Comparison of CD spectra of 80 μM (nucleotide) d(GCG-ATTA-GCG)•d(CGC-TAAT-CGC) (panel A) and d(GCG-AAGTT-GCG)•d(CGC-AACTT-CGC) (panel B) in the absence and in the presence of varying amounts of DST.

Figure 2. Comparison of difference CD spectra (where the DNA spectral contributions have been subtracted out) of 80 μM (nucleotide) d(GCG-ATTA-GCG)•d(CGC-TAAT-CGC) (panel A) and d(GCG-AAGTT-GCG)•d(CGC-AACTT-CGC) (panel B) in the presence of varying amounts of DST.

Figure 1


(A)
d(GCG-ATTA-GCG)[D]
θ (mdeg)
20
10
0
-10
-20
230
250
270
290
310
330
350
370
WAVELENGTH (nm)
8.59 uM
5.16 uM
3.44 uM
1.72 uM
0 uM DST

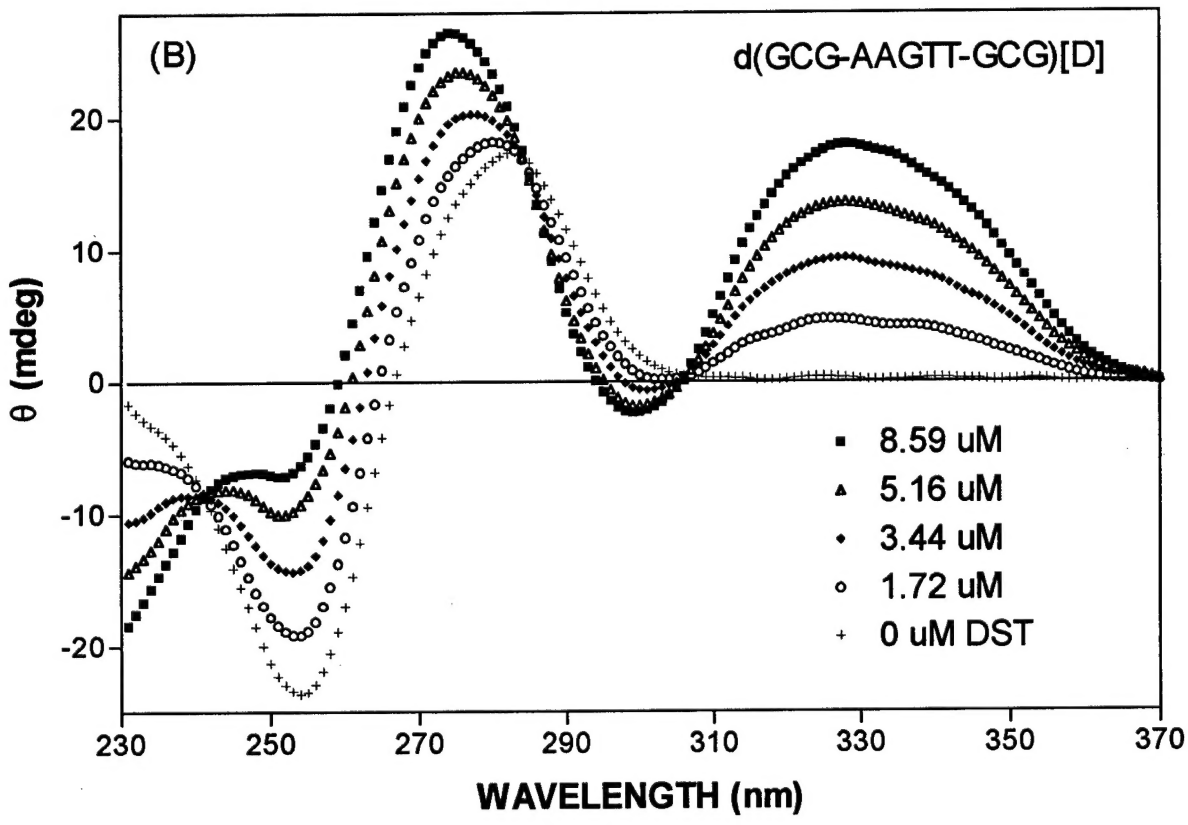
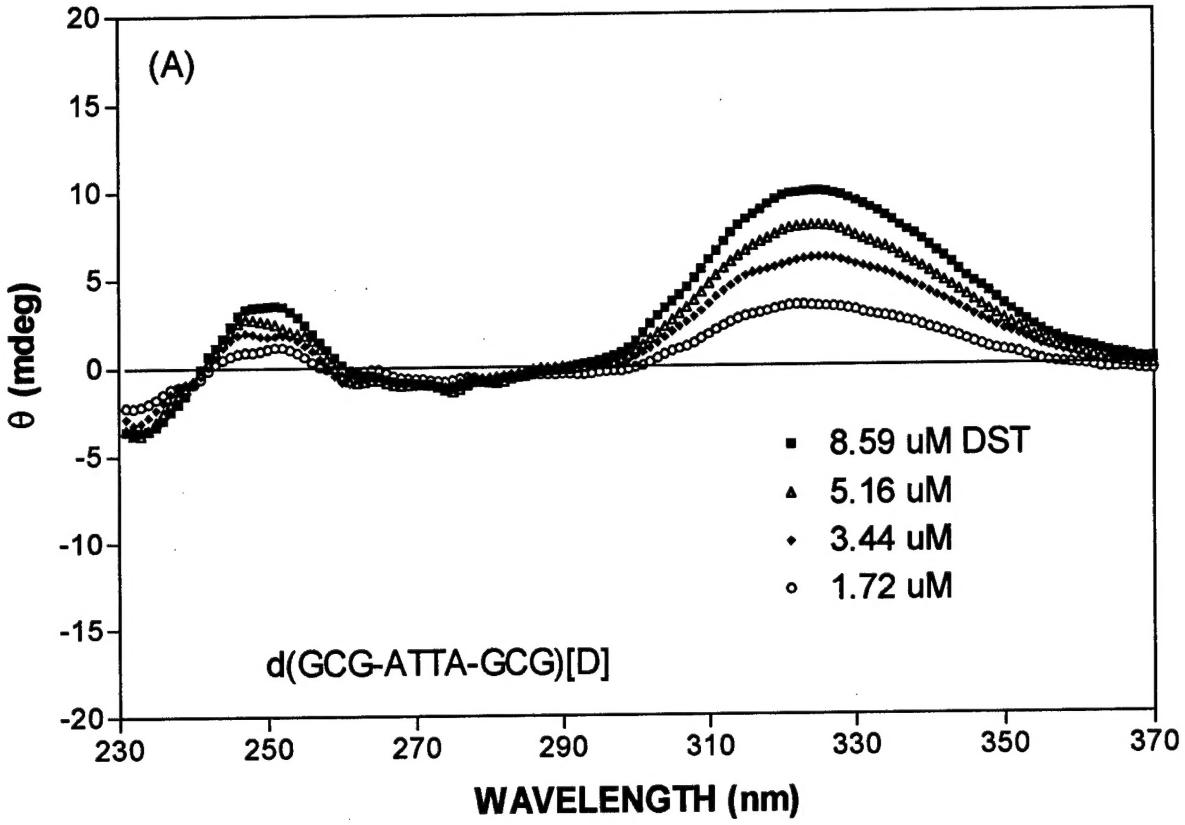
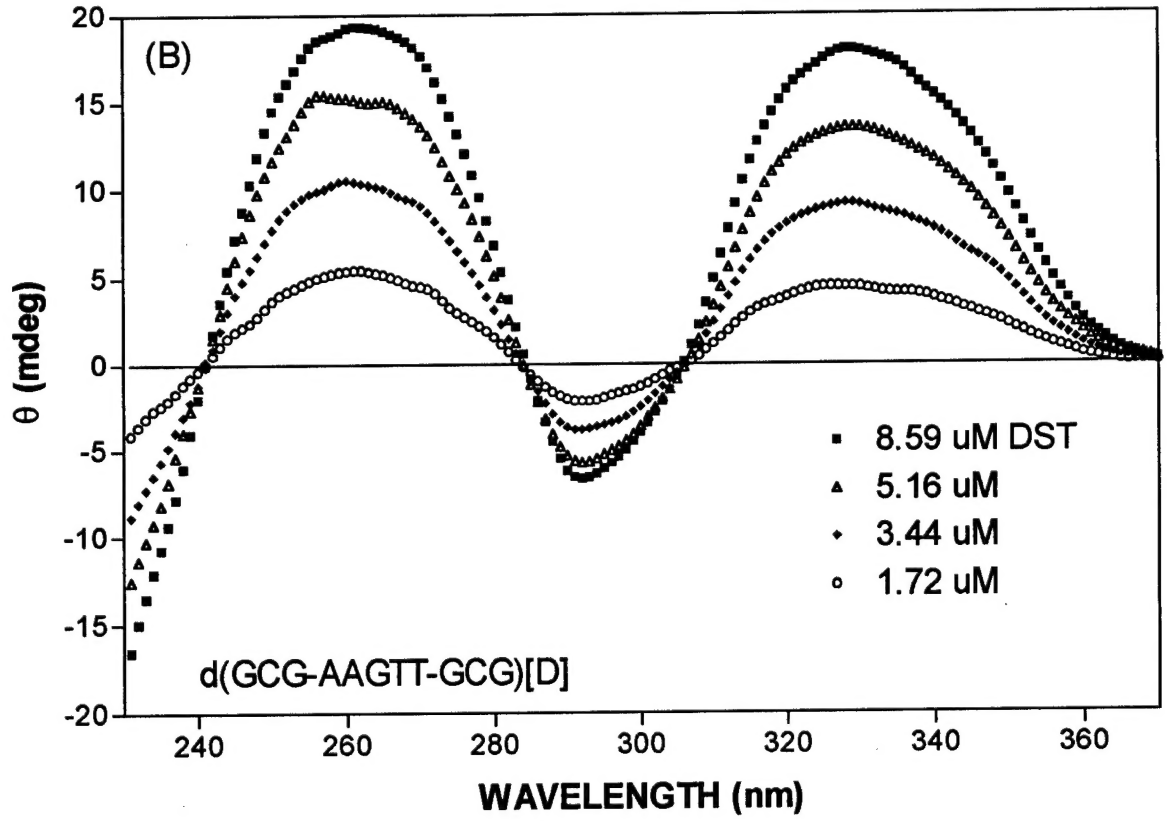

(B)
d(GCG-AAGTT-GCG)[D]
θ (mdeg)
20
10
0
-10
-20
230
250
270
290
310
330
350
370
WAVELENGTH (nm)
8.59 uM
5.16 uM
3.44 uM
1.72 uM
0 uM DST

Figure 2


(A)
θ (mdeg)
WAVELENGTH (nm)
8.59 uM DST
5.16 uM
3.44 uM
1.72 uM
d(GCG-ATTA-GCG)[D]


(B)
θ (mdeg)
WAVELENGTH (nm)
8.59 uM DST
5.16 uM
3.44 uM
1.72 uM
d(GCG-AAGTT-GCG)[D]